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THE ACIDFAST ACTINOMYCETES

WITH A REPORT OF A CASE FROM WHICH A NEW SPECIES WAS ISOLATED

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Acidfast actinomycetes are not common and therefore not as well known as other pathogenic varieties. They are, however, of great interest from the standpoint of bacteriology, since they furnish the closest contact between the true bacteria and higher fungi, being closely related to the tubercle bacillus in morphologic and cultural characters, pathogenicity and serum reactions; and, second, from the standpoint of clinical medicine, since they produce infections that bear a great resemblance to tuberculosis, with which they may be confused, the confusion extending even to the microscopic examination of the sputum.

These infections differ from tuberculosis, however, in having a distinctly more unfavorable prognosis, the organisms being more virulent both for man and laboratory animals.

Acidfast actinomycetes form a well defined group containing several species which resemble each other closely, but may be distinguished from each other by differences in cultural characters and pathogenicity for animals. They may be distinguished from the organisms of lumpy-jaw and ordinary actinomycosis in man because they do not form clubs at the extremity of the mycelium in infected tissues, either in man or ordinarily in laboratory animals (athough under certain circumstances, when the virulence is low, or in certain tissues, as in the kidneys, ray-like masses with clubs at the periphery have occurred in animals inoculated by Nakayama,[1] MacCallum,[2] and Lubarsch[3]). The acidfast actinomycetes may also be distinguished by their acid-fastness, by the readiness with which they can be cultivated, and by their cultural characters; and by their pathogenicity for laboratory animals, especially guinea-pigs.

In morphology, the various species resemble each other closely. The acidfast property was not recognized by the various bacteriologists who

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[1] Arch. f. Hyg., 1908, 58, p. 207.

[2] Centralbl. f. Bakteriol., I, 1902, 31, p. 529.

[3] Ztschr. f. Hyg. u. Infektionskrankh., 1899, 31, p. 187.

first described these organisms, and was apparently first observed by Berestneff,[4] and has been extensively studied by Feistmantel.[5] Fuchs[6] found a number of species to be at least in part acidfast. In sputum the fragments of mycelium may readily be demonstrated by the ordinary stains for tubercle bacilli; they are, however, less acidfast than tubercle bacilli, and may be decolorized if the acid is allowed to act too long. In tissues they cannot be readily demonstrated by the Ziehl-Neelson stain. Very young cultures are not acidfast, a condition also observed in cultures of tubercle bacilli; while in old cultures only certain fragments retain the stain. Feistmantel believes the acidfastness bears no relationship to fats, as is the case with tubercle bacilli.

In their cultural characters they also resemble each other closely, producing on solid medium a dry, wrinkled growth varying from a chalky white through the shades of yellow to a deep orange color, the latter appearing exactly like a culture of tubercle bacilli. The pigment depends in part on the species, in part on the nature of the culture medium and in part on the age of the culture. In liquid medium they form a wrinkled pellicle, such as is produced by tubercle bacilli. With certain exceptions to be noted later, they do not produce active ferments against either proteins or carbohydrates. They grow most luxuriantly on rich mediums containing meat extract and carbohydrates, but show some growth on practically all mediums, some varieties finding nutrition even in tap water.

It is probable that the acidfast actinomycetes occur as saprophytes in soil or on vegetable matter, as is the case with other members of this genus, and that infection occurs through spores that gain entrance to the body by inhalation or in wounds. We have found no record, however, of members of this group being isolated from such sources. Fortineau and Soubrane[7] have described a variety of actinomycetes, isolated by Rappin from the filter beds in Nantes, which was acidfast. It produced a rose to vermilion pigment and produced localized nodules at the point of injection in guinea-pigs. One of us cultivated an organism apparently identical with this from an agar plate exposed to air; this organism was kept for some years without its acidfastness being suspected.

Several species have been isolated from diseases in domestic animals. These are A. farcinica (Nocard)[8] producing a disease of cattle, A. caprae (Silberschmidt)[9] isolated from a lung abscess in a goat, A. canis (Levy)[10] producing infections in dogs, and an unnamed variety isolated from a guinea-pig by Zimmermann.

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[4] Dissertation, Moscow, 1897, quoted by Schabad.

[5] Centralbl. f. Bakteriol, I, 1902, 31, p. 433.

[6] Centralbl. f. Bakteriol., I, 1903, 33, p., 649.

[7] Proc. Congrés International de la Tuberculose, 1905, 1, p. 177.

[8] Ann. de l'Inst. Pasteur, 1888, 2, p. 293.

A. farcinica (Nocard)[8] produces a disease of cattle known as "farcin du boeuf," which occurs sporadically in France and is apparently endemic on the island of Guadeloupe in the French West Indies. It begins as a chronic indurative lymphangitis and lymphadenitis of the subcutaneous tissues, usually on one of the extremities, the lesions developing into cold abscesses which break through, forming sinuses. It is of long duration, but leads to general wasting of the animal, eventually to pulmonary involvement and finally to generalized metastases and death. Cultures were easily obtained on all mediums. On solid mediums a pale yellowish-white wrinkled growth occurred, the surface being dry and powdery. Growth in broth occurred, chiefly at the bottom of the tube in the form of whitish granules, though some colonies floated on the surface like "globules of fat on cold soup." There was no growth on gelatine at room temperature, and no change in milk. Inoculated intraperitoneally into guinea-pigs, death occurred in from 9 to 20 days; the peritoneal surfaces were found studded with miliary nodules, the omentum particularly being a tangled mass of nodules. Injected intravenously, there was produced a condition resembling generalized miliary tuberculosis. Injected subcutaneously, the organisms formed a local abscess which drained for a long time; but extension of the infection never occurred. The cow and sheep proved more resistant, but succumbed eventually. Rabbits, cats, dogs, the horse and ass were not susceptible.

The acid-fastness of this species was apparently first demonstrated by Berestneff,[4] who compared it with other varieties from human sources. It is noteworthy that subcultures of the original strain have maintained their pathogenicity for a long time, guinea-pigs being susceptible after 14 years (Feistmantel),[5] while miliary nodules were produced in monkeys after nearly 20 years (Musgrave and Clegg),[11] computing from the dates of publication.

A. caprae (Silberschmidt)[9] was isolated from a lesion in the lung of a goat, diagnosed as tuberculous by the veterinarian from whom Silberschmidt obtained the culture. It appeared in the form of long, tangled, branching threads, which stained readily by the more intense aniline dyes in smears, but were not readily stained in sections. In cultures the threads soon disintegrated into short bacillary and coccoid forms. On agar small dry, warty, whitish brown colonies were produced which early became covered by a whitish powder; on potato the growth was similar. On broth small white colonies in the form of concave disks were formed, which ultimately coalesced to form a thick pellicle. On milk a pellicle with a rosy tint occurred; the milk remained unchanged. Gelatine was not liquefied. Rabbits were not very susceptible, only one small animal dying after intravenous inoculation; if killed some days after inoculation, miliary nodules were found throughout the body; microscopically these resembled tubercles, giant cells being found in some. Guinea-pigs died in 4 to 6 days when inoculated intraperitoneally; miliary nodules were found on the peritoneal surfaces. Mice were not susceptible.

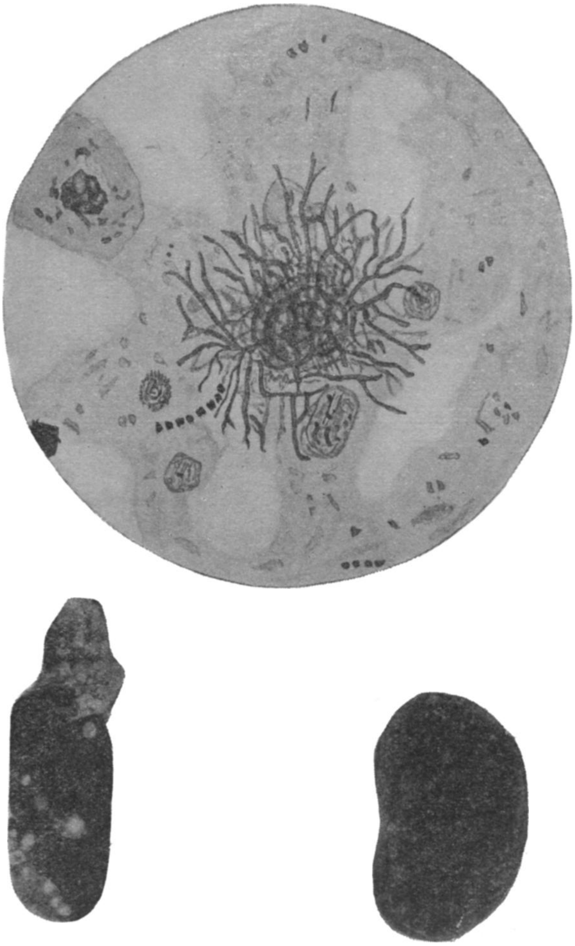
Silberschmidt[9] made a comparative study of his organism with various other actinomycetes, and believed it most closely resembled A. farcinica, differing,

[9] Ann. de l'Inst. Past., 1899, 13, p. 841.

[10] Centralbl. f. Bakteriol., I, 1899, 26, p. 1.

[11] Phil. Jour. Sc., 1907, 2 B, p. 477.

however, in the earlier fragmentation of the mycelium, the growth on the surface of broth, and the growth on milk. In a comparative study with subcultures some years later Musgrave and Clegg[11] found these characters constant. The acidfastness of A. caprae was apparently first discovered by Fuchs.[6]



Figs. 1, 3, 4

Fig. 1.—Mass of mycelium in sputum stained by Ziehl-Neelson method. Camera lucida.
Fig. 3.—Pseudotubercle on surface of spleen. Guinea-pig inoculated intraperitoneally.
Fig. 4.—Miliary abscesses on surface of kidney. Rabbit inoculated intravenously.

Musgrave and Clegg[11] obtained miliary nodules in the peritoneum of monkeys with subcultures.

Rabe[12] found, in two cases of phlegmon and one of peritonitis, in dogs, a filamentous fungus which he named Cladothrix canis. Cultures were not made.

[12] Berlin Tirrarztl. Wchnschr., 1888 (quoted by Levy.)[10]

Levy[10] believed this organism to be identical with Discomyces canis described previously by Rivolta, and with an organism, isolated in his laboratory by Lange and Manasse, which he named A. canis. The latter was obtained from a suppurative phlegmon of the neck in dogs, and proved pathogenic on reinoculation into dogs. Musgrave and Clegg studied a subculture of this organism obtained from Levy. They found it acid fast and pathogenic for monkeys and guinea-pigs, producing miliary tubercle-like nodules on the peritoneum. In its cultural characters it proved identical with A. caprae.

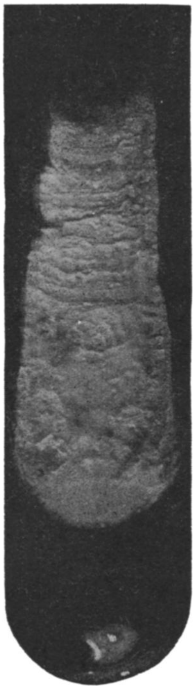


Fig. 2.—Culture on Sabouraud agar 5 days old.

Trolldenier[13] observed an infection in a dog which resembled many of the human cases, the primary infection being in the peribronchial lymph nodes which were caseopurulent, the dog dying from secondary brain abscesses. Smears showed acidfast branching filaments. Cultures were successful, the colonies on agar being dry and white. He believed his strain to be closely related to, but not identical with, A. asteroides (Eppinger[14]).

Zimmermann[15] obtained a culture from the caseous nodules on the peritoneal surfaces of a guinea-pig previously inoculated with a myxomycete (Plasmodiophora carassibe). It grew in the form of white, sugary colonies on agar, which later turned yellow and finally rusty. The growth on potato resembled that of the tubercle bacillus. Smears showed acidfast, branching filaments. It proved pathogenic for dogs, rabbits, guinea-pigs, mice, chickens and frogs. Inoculated intravenously, it killed rabbits in 3 to 5 days; intraperitoneal injections killed guinea-pigs in 4 to 6 days. The peritoneal surface was studded with pseudotubercles which contained no giant cells. He believed his strain was similar to Eppinger's[14] A. asteroides, but that it differed in certain particulars, especially in its greater virulence.

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[13] Ztschr. f. Tiermed., 1903, 7, p. 81.

[14] Beitr. zur path. Anat. (Ziegler's) 1890, 9, p. 287.

[15] Pester med-chir. Presse, 1903, 39, p. 704.

Several varieties of acidfast Actinomycetes have been isolated from a number of cases in man. The first of these was isolated and reported by Eppinger.[14] He named his organism A. asteroides. Eppinger's organism has been considered the type species of the group, and has been extensively studied. A number of cases have subsequently been reported, from which organisms have been isolated which were identical with A. asteroides, while from others have been obtained similar acidfast actinomycetes that differed in certain respects from Eppinger's original strain, and which should be considered as separate species; these, however, have not received specific names.

Eppinger's patient was a man 52 years old, a glassworker, who had rheumatic symptoms, but who soon became delirious and died after two weeks. The clinical diagnosis was meningitis. Necropsy revealed multiple brain abscesses with meningitis, caseous and calcareous peribronchial lymph nodes, and miliary "pseudotuberculosis" of the lungs and pleura; there were diverticula of the esophagus.

From the brain abscesses and meningeal pus the organism was isolated in pure culture. It was found microscopically in decalcified sections of the bronchial lymph nodes. In smears it appeared as long tortuous gram-positive filaments, showing branching which Eppinger considered "false," and coccoid and bacillary forms which showed motility. It has since been shown that Eppinger was in error regarding the false branching and motility.

Cultures were readily obtained on ordinary mediums. In agar the colonies appeared after a day or so as small star-like masses on account of the radiating filaments. Because of the apparent false branching and the star-like colonies, Eppinger named the organism Cladothrix asteroides. It has since been generally known as Streptothrix Eppingeri, but should be correctly termed Actinomyces asteroides. On solid mediums the growth appears as a wrinkled, dry mass, at first whitish, but rapidly turning yellow and later orange, old cultures on potato becoming brick red. On older cultures there appears a white, powdery layer formed by the spores. The growth on solid mediums, especially on dextrose or glycerine agar, bears a close resemblance to that of tubercle bacilli. On liquid mediums a thick, wrinkled, yellowish-orange pellicle is formed. Gelatine is not liquefied, and there is no change in litmus milk.

Rabbits inoculated intravenously died in 5 to 7 days with multiple abscesses throughout the body. Guinea-pigs inoculated intraperitoneally died in from 5 to 24 days, showing multiple miliary "pseudotubercles" on the peritoneal surfaces.

Other cases in which Eppinger's organism was the cause of disease, primary in the lungs, have been reported by Aoyama and Miyamoto,[16] Horst,[17] Schabad,[18] MacDonald,[19] Stokes,[20] and Loehlein.[21]

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[16] Mitt. a. d. Med. Fac. d. Kan. Univ., Tokyo, 1900, p. 231.

[17] Ztschr. f. Heilkunde, 1903, 14, p. 157.

[18] Ztschr. f. Hygiene u. Infektionskrankh., 1904, 47, p. 41.

[19] Scottish Med. & Surg. Jour., 1904, 14, p. 305.

[20] Am. Jour. Med. Sc., 1904, 128, p. 861.

[21] Münch. med. Wchnschr., 1907, 54, p. 1523.

Aoyama and Miyamoto's[16] patient was a Japanese pawnbroker, 35 years old. Illness began with symptoms of an apoplectic stroke. Two months later a cough developed, and after a week's illness death occurred. Necropsy revealed a caseous pneumonia with an abscess cavity and an aneurism of the anterior communicating artery. Sections of the abscess wall showed a small round cell infiltration, but no giant cells. Acidfast actinomycetes were found in smears from the sputum and from pus in the abscess cavity obtained postmortem. None was found in sections of the aneurism which was probably an accidental lesion.

Cultures were readily obtained. On solid mediums the growth was at first grayish, but soon became chalky-white, later turning yellow. On liquid medium a pellicle formed. Growth on gelatine was very poor. No change occurred in milk. Guinea-pigs died in from 4 to 20 days, depending on the dosage and mode of injection. They developed a pseudotuberculous peritonitis, or a hemorrhagic-fibrinous serositis.

The authors made extensive studies of the morphology and resistance of their organism. They found it gram-positive and acidfast. Methylene blue stained the protoplasm but left the membrane clear. Irregularities in the staining of the protoplasm were due in part to plasmolysis, and in part to the presence of chromatin granules. Older cultures underwent fragmentation, and the bacillary fragments were more resistant to heat and drying than the young mycelium.

The organism did not lose its virulence for guinea-pigs after long cultivation.

Aoyama and Miyamoto[16] also observed another case of caseous pneumonia in which an acidfast filamentous organism was present. A complete description, which they promised, was apparently never published.

Horst[17] observed multiple brain abscesses following suppuration of a peribronchial lymph node in a carpenter aged 33. There was also a tuberculous ulcer at the ileocecal valve and caseous mesenteric lymph nodes. The cultures obtained resembled closely those of Eppinger and of Aoyama and Miyamoto. The organism was pathogenic for guinea-pigs, less so for rabbits, and not at all for mice.

Schabad's[18] patient was a man 62 years old, a worker on a ship used for the transportation of grain. He suffered from a cough of 3 years' duration, and had had bloody sputum 6 months previous to admission. He was admitted to the hospital for a tumor mass on the right side of the chest, which proved to be a cold abscess. Acidfast branching filaments were found in sputum and in aspirated pus. Death occurred 5 days after admission.

Necropsy revealed that the infection had begun in a peribronchial lymph node, and was followed by a pulmonary abscess. The latter had broken into the pleura, producing an empyema, and had then eroded the third rib and produced a massive subpectoral abscess.

In its cultural characters the organism was identical with Eppinger's original strain. The results of animal inoculations were also similar to those reported by Eppinger. Pigeons were not susceptible.

MacDonald's[19] patient was a charwoman 65 years old. She had been sick for half a year, was admitted in coma and died the next day. A complete history was not obtained. Necropsy revealed a caseous pneumonia with cavity formation in the lower lobe of the left lung, and secondary abscesses in the lungs, kidneys and brain. Miliary nodules were present on the pleurae. Branching filaments were found in sections of all the lesions; they could not be demonstrated by Ziehl's stain, but stained readily with Gram's method. Sections of recent lesions in the lungs showed bronchopneumonia, the exudate being composed of fibrin and pus cells; in the older areas there was some fibrosis, with mononuclear leukocytes, but no giant cells.

In cultural characters and pathogenicity for laboratory animals the organism was identical with Eppinger's[14] strain. Young cultures were not acidfast, but older cultures undergoing fragmentation were.

Stokes[20] observed a pulmonary infection with A. asteroides in a male infant 28 days old. There was an abscess cavity the size of a pea in the lower lobe of the left lung. Smears showed acidfast branching filaments, and cultures were obtained, which proved to be identical with Eppinger's organism in all particulars.

Loehlein[21] reported a case of multiple brain abscesses, with exudate in the ventricles, in a man 58 years old. There was also bronchiectasis and diseased peribronchial glands, and although the fungus could not be demonstrated in the latter, Loehlein believed that they were the site of the primary infection. The organism isolated was like the A. asteroides. It was pathogenic for rabbits and guinea-pigs, but not for dogs.

Three other cases of infection with A. asteroides have been reported, in which the primary infection was elsewhere than the lungs. Two of these were primary infections of the peritoneum following laparotomy, the third being a case of Madura foot.

MacCallum's[2] patient was a child of 3 years old suffering from stricture of the esophagus following the accidental swallowing of lye. Three weeks after a gastrostomy the child died of generalized peritonitis. A. asteroides was isolated in pure culture. This strain proved pathogenic for rabbits, dogs and guinea-pigs.

Rabbits were less susceptible than guinea-pigs, living 10 days or more following intravenous injection. In some of the abscesses formed in these rabbits the fungus was arranged in irregular masses about a core of calcium carbonate; the peripheral filaments of these masses showed clubbed extremities. Giant cells occurred in some of the lesions in rabbits.

Foulerton[22] obtained his culture from the peritoneal exudate of a man 55 years old on whom an appendicostomy had been performed for the purpose of feeding; the patient was suffering from a carcinomatous stricture of the esophagus. There was a retrocecal abscess and a secondary abscess in the lung. Death occurred 24 days following operation.

These two cases show a remarkable parallelism. In both the peritoneum was healthy at the time of operation; the organisms then must have been introduced during the operation, probably from spores floating in the air. It is a noteworthy coincidence that both cases were suffering from esophageal strictures. Eppinger's original case also showed esophageal stricture with diverticula.

A case of Madura foot has been reported by Musgrave and Clegg.[11] The patient was a Philippino woman, 30 years old. The infection was of 3 years' duration. Amputation was followed by recovery. In the pus the organism appeared as yellowish granules, which when crushed and examined unstained failed to show the clubbed extremities characteristic of the Madura foot parasite. Its cultural characters were similar to those of A. asteroides. It proved pathogenic for dogs, monkeys and guinea-pigs; rabbits and pigeons were not susceptible. Injected into the foot of a monkey, a typical mycetoma developed.

The authors named this organism Streptothrix freeri, but in a later paper recognized its identity with A. asteroides.

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[22] The Lancet, 1910, I, pp. 551, 626 and 769.

In all of these cases the organisms were practically identical in all features. The strain isolated by Aoyama and Miyamoto[16] differed in that the growth was chalky white and only turned yellow after some time, never becoming orange. With all of these strains the guinea-pig showed the greatest susceptibility, the rabbit being next; dogs were infected by some strains, not by others. None of the strains liquefied gelatine or digested the casein in milk.

Other cases have been reported, however, in which the organisms isolated differed in some respects from the variety described by Eppinger.

Birt and Leishman[23] cultivated an acidfast filamentous organism from the pleural pus of a South African soldier. The patient, 26 years old, became ill during the siege of Ladysmith and died 6 months later, 11 days following resection of a rib for empyema. Acidfast rods and segmented branching filaments were found in the sputum, and the organism was isolated in pure culture from pus aspirated from the chest. Necropsy revealed a chronic bronchopneumonia, without cavity formation, in the right lung. Both lungs were studded with small cirrhotic nodules.

The colonies in agar appeared like small balls of cotton. On agar slants the growth was at first snow-white, later becoming a delicate coral pink color. Gelatine was not liquefied, but milk was slowly peptonized. On broth a pellicle was formed. Guinea-pigs inoculated intraperitoneally died in 5 to 6 weeks with caseous masses in the omentum.

This strain differs from A. asteroides in the absence of orange pigment, the peptonization of milk, and the lower virulence for guinea-pigs. Birt and Leishmann also found that their strain would grow on ordinary tap water, whereas Eppinger's strain did not.

Bernstein's[24] organism was characterized by a pure white growth, which on solid mediums formed hard masses and on liquid mediums a thick pellicle adherent to the walls of the culture tube. It did not liquefy gelatine; the action on milk is not described. It was very pathogenic for rabbits and guinea-pigs, one of the former developing endocarditis following intravenous injections, the latter developing lesions in the testicles in addition to miliary nodules on the peritoneal surfaces. Mice were not susceptible.

This organism was isolated from a suppurating localized pneumonia in the lower lobe of the right lung of a carpet worker aged 67. There was also generalized miliary tuberculous peritonitis; sections of the nodules on the peritoneum showed typical tubercles with acidfast bacilli. Bernstein is unable to state whether these were true tubercle bacilli or fragments of mycelium.

A third variety comprises the strains isolated by Ferré and Faguet,[25] Sabrazés and Riviére,[26] Berestneff,[4] and Scheele and Petruschky.[27] These differed from those previously described in that they liquefied gelatine and were nonpathogenic for laboratory animals; some of them grew anaerobically.

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[23] Jour. Hyg., 1902, 2, p. 120.

[24] Proc. Roy. Soc. of Med., 1908-1909, 2, P. 3, p. 271.

[25] Semaine méd., 1895, p. 359.

[26] Semaine méd., 1895, p. 383.

[27] Verhandl. d. 15. Cong. f. inn. Med., 1897, p. 550.

Ferré and Faguet isolated a branched filamentous organism from a brain abscess. The cultures on solid mediums were gray-white without any whitish bloom on the surface. Neither rabbits nor guinea-pigs were susceptible.

Sabrazés and Riviére[26] saw two cases, the first a brain abscess with a suppurating infarct in the kidney, the second a case of bronchopneumonia with multiple miliary subcutaneous abscesses. The organisms from these two cases were identical. The growth on solid medium was yellowish. They grew best under aerobic conditions, but showed some growth anaerobically. Gelatine was liquefied. They were not pathogenic for laboratory animals, but showed some pathogenicity when lactic acid was added to the material injected.

Berestneff's[4] patient died of multiple cerebral abscesses, one of which had perforated into the lateral ventricle. There was a cavity in the upper lobe of the left lung, with a bronchopneumonic area in the lower lobe, and metastatic abscesses occurred in the skin. The organism was found in all of these lesions. The culture liquefied gelatine, and was practically nonpathogenic, although when injected subdurally into guinea-pigs, miliary nodules were produced on the meningeal surfaces. Berestneff considered his strain to be identical with those of Ferré and Faguet, and Sabrazés and Riviére.

Scheele and Petruschky[27] reported a case of fatal pleuropneumonia with secondary abscesses in the skin, in a woman aged 56. Acidfast branching filaments were found in the sputum and in pus from the dermal abscesses during life. The organism produced a firm white growth on agar, better on dextrose agar, and did not grow at all on glycerol agar or potato. In a communication to Schabad the authors reported that it slowly liquefied gelatine, and produced only slight localized abscesses in guinea-pigs.

In addition to these cases, a number have been reported in which acidfast actinomycetes have been demonstrated in lesions or exudate, but in which it was impossible to obtain cultures.

Ljubimoff, in a personal communication to Berestneff,[4] reported a case of fatal bronchopneumonia which he observed in 1888, in which long acidfast branching filaments were found in the sputum during life. Berestneff succeeded in demonstrating their presence in the sections of the lung removed postmortem.

Flexner's[28] patient was a man of 70. The symptoms were those of pulmonary tuberculosis. No sputum was obtained. Necropsy revealed a caseous bronchopneumonia of both lungs, with beginning cavity formation in places and miliary pseudotuberculous peritonitis. Smears from the exudate showed acidfast branching filaments. Cultures remained sterile. A guinea-pig inoculated with the exudate died after some weeks without demonstrable lesions.

Buchholz[29] observed a fatal case of empyema following bronchopneumonia with lung abscess in a steel worker, 38 years old. Branching filaments were found in the exudate, but streptococci were present as well. He believed the organism was similar to A. asteroides.

Musser[30] reported a case of fatal bronchopneumonia in a man of 37. Acidfast rods and long branching filaments were found in the sputum. Cultures were unsuccessful. Guinea-pigs inoculated with sputum died from Streptococcus infections.

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[28] Jour. Exper. Med., 1898, 3, p. 435.

[29] Ztschr. f. Hyg. u. Infektionskrankh., 1897, 24, p. 470.

[30] Proc. Philadelphia Co. Med. Soc., 1901, 22, p. 245.

Butterfield's[31] case was that of a young man who died of diabetic coma. He had had a cough for some time. Tubercle bacilli were not found in the sputum. At necropsy a consolidated area with a central cavity in the right upper lobe was found. Smears showed acidfast branching filaments.

Ribytkof and Maloletkof reported a case of spinal cord abscess with meningitis in which acidfast actinomycetic mycelium was demonstrated. No portal of entry for the infection was found.

Foulerton[22] found acidfast mycelium in the sputum of a man 50 years old who had suffered from pulmonary disease for a year. The organism could not be cultivated and proved nonpathogenic for guinea-pigs. Foulerton also reported another case personally communicated to him, in which similar structures were found in the sputum; the patient was a man of 28, who developed pulmonary disease following the removal of what was believed to be a tuberculous kidney. The outcome of these two cases is not reported.

The case that we report is of interest because the condition was diagnosed during life; the organism was isolated in pure culture from the sputum and proved to be different from any of the recorded species; immunologic studies were made and vaccine treatment was tried.

The patient was a woman of nervous type, 31 years old, married, who had two children. She complained of cough, periodic expectoration on lying down, and general weakness. About 3 years ago, following childbirth, she took cold and began to cough. She had never been well since, having frequent attacks of tonsillitis and a chronic cough. At first there was very little sputum; what little there was "appeared greenish and shiny, with a peculiar medicated odor." But about 2 years ago she began coughing up large quantities of sputum, a cupful or more during the day. The sputum was occasionally blood streaked. She gradually developed a goiter for which an operation was performed one year ago. Three months later she began to cough more and had severe pain in the right side of the chest. There was fever for several days at this time, and for several months there was a degree of fever every day or so.

She was first seen July 18, 1919. Physical examination at this time revealed nothing noteworthy save an area of impaired resonance with crackling râles in the right axillary region from the fifth to the eighth ribs. Roentgen screen examination showed slight thickening of both apexes and small annular shadows near the hilum of the right lung. There were no areas that suggested massive consolidation or large accumulations of pus.

The laboratory findings were negative with the exception of the sputum, which will be described later. The temperature showed no abnormal variations during two weeks.

Intracutaneous tuberculin tests were positive both with Koch's O. T. and tuberculin prepared from tubercle bacilli treated with CO_2 under high pressure, according to the method of Larson,[32] producing areas of infiltration the size of a half-dollar in 48 hours.

The sputum was examined for the presence of tubercle bacilli by the Ziehl-Neelson method. The examination revealed the presence of acidfast branching filaments. For the most part these were isolated strands showing lateral branches at irregular intervals, but occasionally there was found a considerable mass of mycelium in which the central filaments were closely intertwined, the peripheral portions being irregularly arranged in a radial fashion, resembling somewhat the sulphur granules of ordinary actinomycosis. For the most part, the filaments were quite uniform in diameter, although occasional fusiform swellings occurred; but definite clubbed extremities were never found. In addition to these structures, there were many mycelial fragments of varying length, some showing a remarkable resemblance to true tubercle bacilli. The mycelium was not uniform in its acidfastness, some strands taking the blue counterstain. Moreover, it was not so acidfast as tubercle bacilli; for, if the 5% hydrochloric acid-alcohol used as a decolorizing agent was allowed to act for a minute or more, practically all of the mycelium was decolorized. Several specimens were examined during the course of 2 weeks, and all showed the presence of this organism, although in some specimens they were much more numerous than in others. There were numerous bacteria of various kinds in the sputum and all attempts to isolate the organism by plating directly from the sputum were unsuccessful, all the plates being overgrown by spreading colonies of bacteria.

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[31] Jour. Infect. Dis., 1905, 2, p. 421.

[32] Jour. Infect. Dis., 1918, 22, p. 271.

Two guinea-pigs were inoculated intraperitoneally with sputum. These died after 5 and 6 days. Necropsy revealed small tubercle-like nodules scattered over the peritoneal surface, being most numerous on the under surface of the diaphragm and the surface of the liver and spleen. The omentum was shrunken to a thick mass in the upper abdomen, studded with nodules. A few flakes of fibrin were present on the peritoneal surfaces, and the parietal peritoneum in addition showed hemorrhages.

From the peritoneal tubercles removed aseptically and crushed, the organism was readily grown in pure culture. The colonies in glycerol agar appeared in 24 hours as small white dots which under the microscope showed the star-like structure described by Eppinger. Subcultures from these colonies grew on all mediums, growth appearing over night.

On agar the growth appears first as a thin grayish veil that soon becomes thick, opaque and chalky-white in color, the surface being dry and wrinkled. The growth is firmly adherent to the medium, and when touched with the inoculating wire breaks off in large flakes, quite unlike the mealy consistence of A. asteroides. If the whitish bloom is scraped off, the underlying mycelium is found to have a buff color, but never assumes the distinct yellow or orange of A. asteroides. We have compared our strain with two subcultures of Eppinger's organism obtained from different sources.

On agar containing various carbohydrates, such as dextrose, maltose, and glycerol, growth is more rapid and more luxuriant than on plain agar, but otherwise it is quite the same. The growth on potato is similar to agar. On Dorsett's egg medium and Loeffler's blood serum, growth is not so rapid. Neither of these mediums are digested.

In gelatine, stab culture growth occurs only on the surface. The medium is liquefied, digestion beginning in a few days and being complete in a week. The liquefaction is stratiform.

In liquid mediums growth occurs only on the surface, appearing first as small white flakes floating on the surface which soon coalesce to form a thick, wrinkled, snow-white pellicle, turning up at the edges to extend a centimeter or more on the glass container. After about 2 weeks this pellicle breaks on jarring and falls to the bottom of the vessel, after which no further growth occurs.

A similar pellicle, having a somewhat yellowish tinge, is produced on litmus milk. The milk turns alkaline and after a few days is curdled, apparently due to the secretion of rennin. At this time the litmus is reduced. The coagulated casein is then slowly digested, peptonization being complete in about 2 weeks.

None of the carbohydrates were fermented. Starch agar plates failed to show any diastatic action. Blood agar showed no hemolysis

All of the meat extract mediums showed some change in color after a time, the mediums becoming darker and assuming a brownish tint. This change also occurred in Dunham's peptone solution, where it was more striking, the colorless fluid first assuming a straw color just beneath the pellicle, the color then gradually diffusing through the medium and after some weeks becoming quite dark.

This brownish discoloration of medium containing meat extract or peptone is quite characteristic of many saprophytic actinomycetes found in soil, and has also occurred in such cultures of the lumpy-jaw organism as we have had an opportunity to study. It has been explained by Beijerinck as being due to the formation of quinone, and is associated with the fermentation of tyrosine. Aoyama and Miyamoto observed a darkening of the mediums with their strain, but otherwise we have found no mention of this reaction with acidfast actinomycetes. It did not occur with the two subcultures of A. asteroides, which we examined.

Our organism grew readily on such simple mediums as Czapek's agar, containing only inorganic salts and saccharose, and agar made from an infusion of soil.

A broth culture several days old was shaken till the pellicle was pretty well broken up, and inoculated in 1 c c quantities intravenously into rabbits and intraperitoneally into guinea-pigs.

The rabbits died in about 48 hours. Necropsy revealed minute abscesses in various viscera, but especially numerous in the kidneys which were studded with minute, yellowish white dots that bulged slightly above the cortex. On section, these were found scattered throughout both cortex and medulla. Microscopic examination revealed them to be simple aggregations of polymorphonuclear leukocytes.

The guinea-pigs died in from 4 to 6 days. The lesions were exactly like those described for the guinea-pigs inoculated with sputum. Sections of the nodules showed them to be small abscesses containing necrotic material and pus cells in the center, and encapsulated by a zone of granulation tissue in which mononuclear leukocytes and proliferating fibroblasts were the only cellular elements.

After 8 months' cultivation, the virulence for animals was undiminished.

Having obtained a pure culture, we prepared an antigen for immunologic studies. The organism was grown in flasks on broth containing 3% each of dextrose and glycerol, until the pellicle broke, after about 10 days. It was then heated to 60 C. for one hour, and placed in Dr. Larson's apparatus under the pressure of a fresh tank of carbon dioxide over night. On releasing the pressure it was found that the masses of mycelium had disintegrated, forming a uniform suspension of mycelial fragments. The organism, however, still grew when subcultivated; the suspension was again heated to 65 C. for an hour, and 0.25% of tricresol added. It was then found to be sterile.

Part of this material was used in this condition, while another portion was passed through a porcelain bougie, and the clear filtrate was used.

Skin tests were made with both of these antigens, injecting 0.1 c c of a 1:1,000 dilution intracutaneously. That night the patient had a chill, the temperature rose to 102 degrees, and she ached all over. The next day the temperature was normal. In spite of this severe constitutional reaction, there was no visible reaction at the point of inoculation after 48 hours.

The antigen prepared as above proved nonhemolytic and not anticomplementary, and was therefore used for complement fixation tests with the patient's serum. The reaction was found to be strongly positive, the complement being completely bound, using 2 drops of the patient's serum and 2 drops of the antigen, and the antisheep hemolytic system ordinarily used in our Wassermann tests. A similar test made with this antigen and the serums of 12 other persons gave negative results throughout.

As the mycelial fragments, after treatment with CO_2 under pressure remained in suspension for some time, the antigen was also useful for agglutination tests. The tests were made by the macroscopic method, incubating at 55 C. for an hour, and read after standing in the icebox over night. The patient's serum agglutinated completely in a 1:20 dilution, and partially at 1:100; a control serum produced no agglutination.

The antigen prepared as described above was used for vaccine treatment, beginning one week after the intracutaneous injections. The vaccine was injected subcutaneously, given at intervals of 4 or 5 days. The first three doses were 0.1 c c, 0.5 c c, and 0.5 c c of the 1:1,000 dilution; the fourth and fifth doses were 0.1 c c and 0.5 c c of the 1:100 dilution, and the sixth dose was 0.1 c c of the 1:10 dilution. After the third dose there was a very slight febrile reaction and a small lump at the point of injection; otherwise, there was no reaction to the injections of vaccine.

After the last injection she returned to her home in another state. Her physician there was furnished with a supply of the vaccine, and the injections were continued for another month. After each injection a very painful lump developed, and the vaccine was discontinued for this reason; no constitutional reactions occurred. The dosage was not further increased.

Letters received from the patient in January and October of 1920 indicate that the disease has progressed somewhat; the cough and expectoration continue, and on 3 occasions there have been hemorrhages from the lungs. Following these hemorrhages there has been fever, but at other times her temperature has been normal. She gained weight for a while and then lost 12 pounds, but has been gaining weight lately. She has complained much of pain in the chest.

Injections of vaccine were continued; the patient was also placed on the usual tuberculosis regime, but otherwise no treatment was given. On returning to her home she again resumed her usual household work.

Infections with acidfast actinomycetes cannot be frequent, as we have been able to find but 26 reported cases. Undoubtedly they are more frequent than is indicated by these reports, however, for some cases have probably been seen which have not been published, and certainly a still greater number have been undiagnosed. The disease shows no particular geographic distribution, cases having been reported from America, England, France, Germany, South Africa, Japan, and the Philippine Islands.

The ages of the patients have varied from 4 weeks to 70 years; with the exception of 2 cases in young children, the ages are fairly evenly distributed throughout adult life. The majority of the cases occur in males. There is considerable evidence that the actinomycoses are occupational diseases, the majority of cases occurring in men who handle grain or straw or similar material, on which the organisms are believed to live. With the acidfast actinomycetes, however, no such condition can be shown, for only one patient in whom the occupation is recorded was a handler of grain. Several patients, however, were engaged in occupations that would be likely to lead to pneumonokonioses. With the exception of 3 cases, 2 of postoperative peritonitis and one of Madura foot, the infection has been primary in the pulmonary tract, although in several cases this has been assumed rather than proved. A survey of the necropsy findings indicates that the sequence is somewhat as follows: The first lesions are in the peribronchial lymph nodes, the organisms probably having been introduced by inhalation. Death may follow metastatic infection from this area without involvement of the lungs, as in Eppinger's case; but more frequently a caseous bronchopneumonia occurs followed by central softening and cavity formation. The infection may remain localized in the lungs, but frequently is accompanied by empyema or by metastases, the latter localizing most frequently in the brain. In several cases there was apparently a hematogenous distribution shortly before death with the production of numerous miliary pseudotubercles.

The duration of the illness varies from 2 weeks to 3 years, with about 6 months as the average. Some cases have been relatively acute.

Microscopically the lesions in man show necrosis with pus cells in the neighborhood of the mycelium. These areas are soon surrounded by granulation tissue containing mononuclear cells, but giant cells do not occur. The lesions in experimentally inoculated animals are quite similar; it is only when strains of attenuated virulence, or resistant animals are used that lesions histologically resembling true tubercles are found. The histogenesis of the lesions in animals has been extensively studied by MacCallum [2] and by Nakayama.[1]

Less than half of the cases have been diagnosed antemortem. The disease is naturally confused with tuberculosis. The diagnosis is established by finding long branching acidfast filaments in the sputum. Frequently a great deal of the mycelium has undergone fragmentation, and bacillary forms may be found in many fields before intact mycelium is seen. At first glance these mycelial fragments resemble tubercle bacilli quite closely, and may lead to erroneous diagnosis. Birt and Leishmann [23] report that they were thus led astray on their first examination of the sputum from their case, and Foulerton believes that many cases have been diagnosed as tuberculosis for this reason. The mycelial fragments in our case, however, showed much greater variation in length than is common with tubercle bacilli.

Zupnik observed that guinea-pigs inoculated with acid-fast actinomycetes gave positive tuberculin reactions, and our patient gave markedly positive von Pirquet tests. We unfortunately failed to test our patient's serum against tubercle bacillus antigens for complement fixation, and also have neglected to test the serums of tuberculous patients against our antigen, so that we are unable to state whether or not complement-fixation tests can be of use in diagnosis. Claypole,[33] however, has observed that guinea-pigs inoculated with acidfast actinomycetes bound complement with tubercle bacillus antigens, and vice versa. It is clear, therefore, that from the standpoint of immunity tests these organisms are very closely related, and if serum reactions are to be used in differential diagnosis, they must be made quantitative.

The prognosis is not good. The case of Madura foot recovered following amputation; in several cases the outcome was not reported; the remainder terminated fatally.

There is nothing in the collected literature to guide us in the matter of treatment. Iodides, which have proved successful in some cases due to other actinomycetes, have apparently not been used in any infections due to the acidfast group. In view of the disastrous results that have been reported following the use of the iodides in pulmonary tuberculosis, it is doubtful that their administration in these infections would be justified. Wynne has reported the successful use of an autogenous vaccine in a case of pulmonary actinomycosis due to a nonacidfast variety. We are unable to state whether or not our patient was benefited by the vaccine. The fact that she was alive a year after the treatment was begun cannot be overlooked when one considers the progressive downward course of the cases hitherto reported.

In summarizing the bacteriologic data that have been published, it is clear that at least three distinct species from human sources have been previously described, ours making a fourth.

A. asteroides (Eppinger) is characterized by a growth of mealy consistence on solid medium, varying from pale yellow to deep orange in color. It does not liquefy gelatine or peptonize milk and is more pathogenic for guinea-pigs than for rabbits. It has been reported more frequently than the other varieties.

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[33] Jour. Exper. Med., 1, 1913, 17, p. 99.

The strain isolated by Aoyama and Miyamoto apparently occupies a position midway between Eppinger's variety and the next species. It differs from A. asteroides only in the whitish color of the growth and the darkening of the medium. It apparently resembles somewhat A. caprae. Bernstein's strain was apparently similar to this one.

The unnamed species described by Birt and Leishman is characterized by a snow-white growth on solid medium. It does not liquefy gelatine but does peptonize milk. It is pathogenic for guinea-pigs; rabbits were not inoculated.

The Berestneff type is characterized by a gray to whitish growth and the liquefaction of gelatine. It is nonpathogenic for laboratory animals.

Our variety resembles the strains of Aoyama and Miyamoto and of Birt and Leishman in the white appearance of the growth on solid media. This whiteness is due to the aerial spores which develop very early; we would characterize it as a chalky white, since the surface is without luster. Our organism is apparently more virulent for laboratory animals than those hitherto reported from man, in this respect resembling most closely the strain isolated by Zimmerman from the guinea-pig; rabbits are distinctly more susceptible than guinea-pigs.

Our strain is particularly characterized, however, by its proteolytic activity, as evidenced by the alkaline coagulation of milk (rennin), the digestion of the coagulated casein, the rapid liquefaction of gelatine, and the darkening of mediums containing peptone (tyrosinase). In this respect it resembles the organism of lumpy-jaw and the saprophytic soil actinomycetes. In fact, we can arrange the various species of acidfast actinomycetes in a fairly uniform series, with Eppinger's variety at one extremity establishing contact with the acidfast bacteria, which it resembles very closely in cultural characters, and our own strain at the opposite extremity, where it joins the nonacidfast actinomycetes. Because of the chalky-white appearance of the growth on mediums, we propose for our new species the name Actinomyces gypsoides.